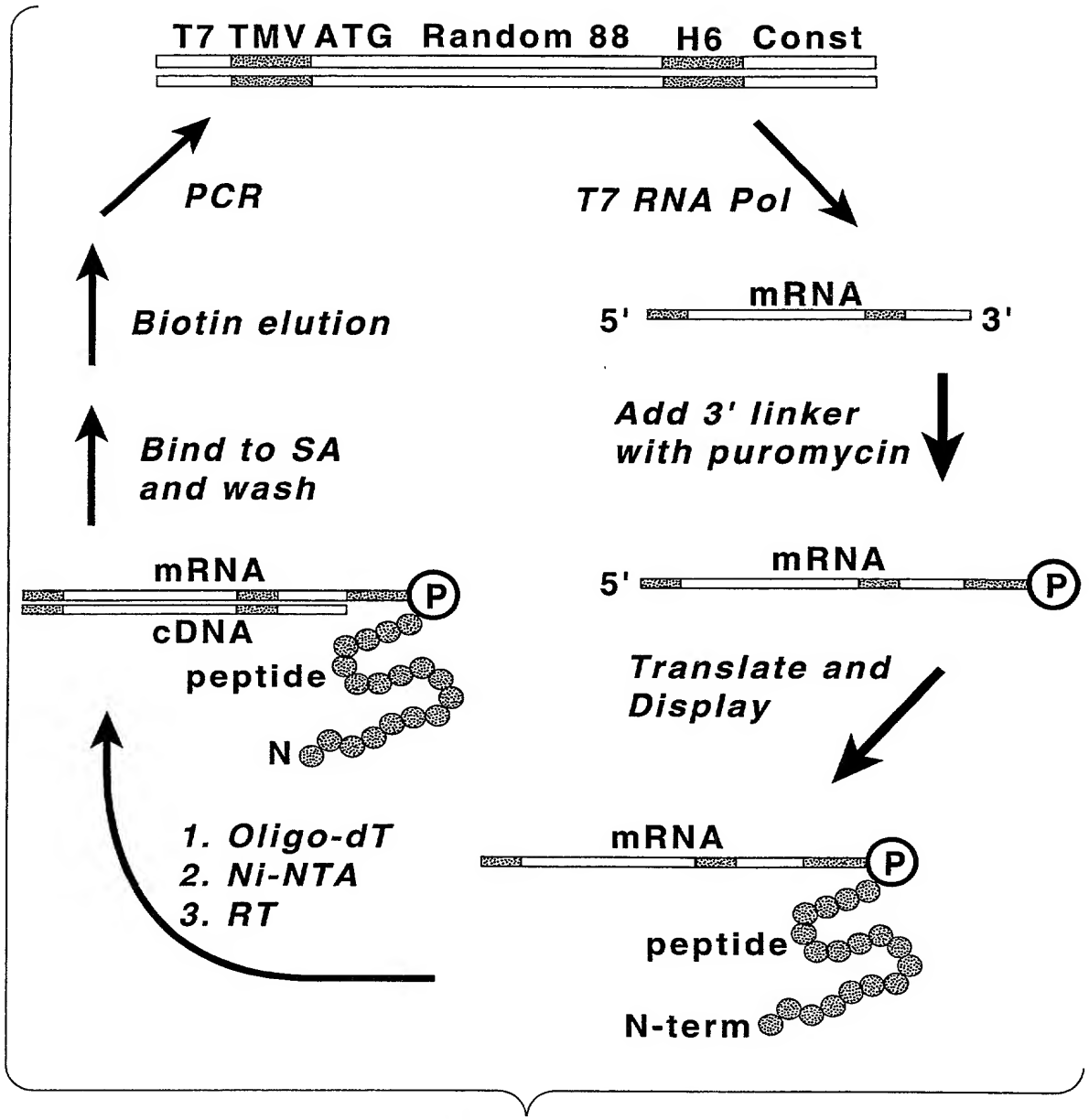
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PCR
T7 RNA Pol
Biotin elution
mRNA
5'
3'
Bind to SA
and wash
Add 3' linker
with puromycin
mRNA
cDNA
P
peptide
N
5'
mRNA
P
Translate and
Display
1. Oligo-dT
2. Ni-NTA
3. RT
mRNA
P
peptide
N-term

Fig. 1A

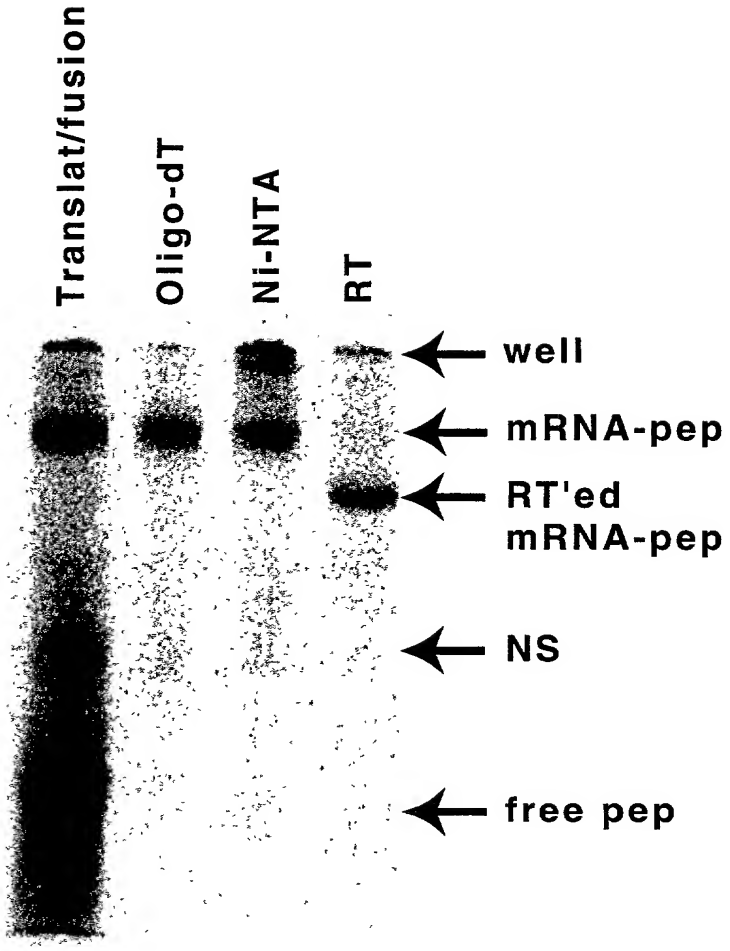
Translat/fusion
Oligo-dT
Ni-NTA
RT
well
mRNA-pep
RT'ed
mRNA-pep
NS
free pep

Fig. 1B

Proof Technology

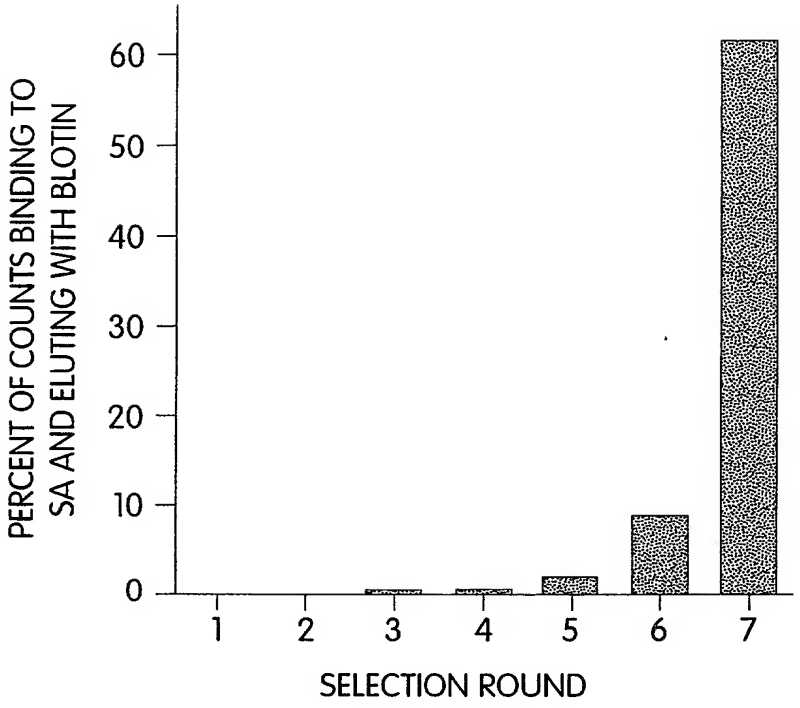

Fig. 2A

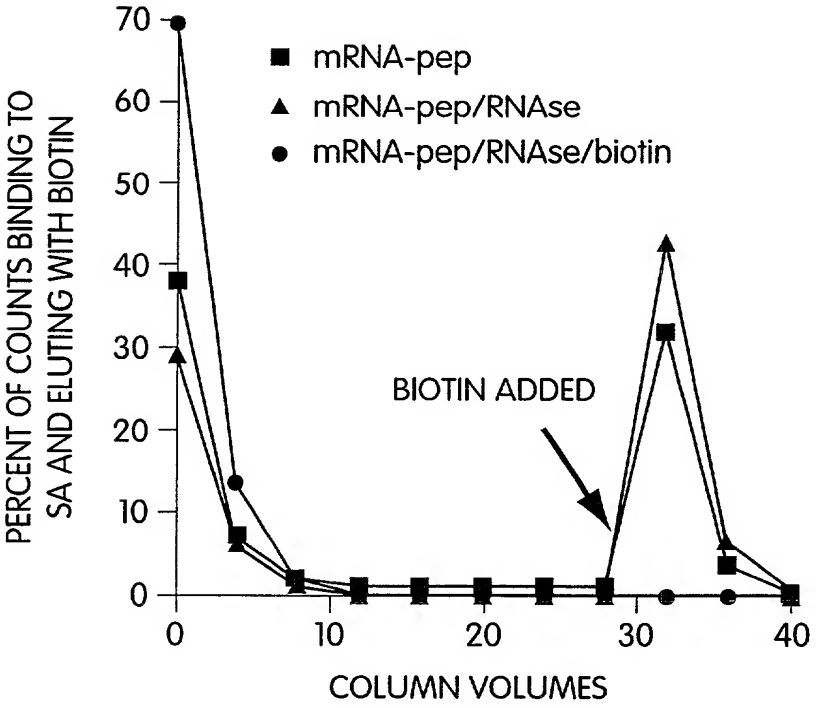

Fig. 2B

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SB2	2	MDEKTHCFHPGDHLVRLVEELQALAEGLQRQGGRQPHRLPRRRPHHLQLLLDEA**HPQ**AGPLRERAHQVDGRLLLQH**HPQ**GDRLLQQPQDHPLELVWRLPPS	2
SB3	4	MTRRPTASSSSCVRHLLLRQGEHGHQALEDRDKARHVRLVEGDVEVLGGLDRLARARHEAL**HPQ**AGLVHLPLHGGDLGGHLRLVLEA**HPQ**GDRLGLAVHHH	3
SB4	1	MDEKTHWGISTWRGEPLLH**HPQ**AGRLPLDRRRARHRRILGAEPGGVDHGLRLELLDDHRPLVPDH**HPQ**RGPLQRGDLPQVVPLVRLRHAHVLGLGLAAATIT	4
SB5	3	MDEKTHWVNVY**HPQ**GDLLVRGHGHDVEALHDQGLHQLDLLVGPPPEVVRALRGEVLGGLRRLVPLD**HPQ**GEALDQARQRPQHLLELHHRALPPALVWRLPPS	5
SB6	1	MDEKTHWLNNFEELLARLDGLREGEDHPLVLRH**HPQ**GDGLLDQPLGRHRALDGEVREGDRPLDQGGEEDLGALVDDDGEVLDGLVHVGVHVHDPLVCGCHHH	6
SB7	1	MDEKTHWFGTLNSFPTHWMSAVGNGKIDCSFNMNLSLNHWLSSGHPDGALDDQL**HPQ**GDALVGRDDGVVQALRLEGQHQHRRLAQRRADRHRQLVWRLPPS	7
SB8	1	MDEKTHCTIELNFSFTHWKLHH**HPQ**GDALLDDGVRPHHPLADEGGGLDQGLGHRRGVVAERLARRDPEVLEGLVERHRGLVPRLRHGGERHAEPLVWRLPPS	8
SB9	1	MDEKTHCNTGLYDGAADCFNELNKDVAPLVEGRHDLVEGLLLER**HPQ**GDPLVAHRQLVHHPLLGRGERHRRALVPQQEHQPHRLQPVVDLGRRRLVWRLPPS	9
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SB11	1	MDEKTHWHERVHHLADGLEQ**HPQ**GQRRPLVERHRQVPRGLVRELQHEGLPLEHPAGVHVIRLHQGDDRDVDGLVDGHGRDVRGLEREVGDGPHRLVWRLPPS	11
SB12	4	MDKDPLLEELEELRERLVH**HPQ**GGLLPLRGQVGHDAERLGAEVDDLRGGLLDEPQRAVAGLHHVPHRVGQRLVHEVRELDEGLLDQRDDLRQRLVWRLPPS	12
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SB17	2	MDEKTHWVGDLQEPLGPLHGGVGEVPGGLVLRH**HPQ**RDRLVDGVGPHGRALARRPHRVVEGLHHLLQRGGERLPPDGPRQLGLLGGELDRADPALVWRLPPS	17
SB18	1	MDEKTHCAVNVNVGLTHWCHRVAHLQPLDP**HPQ**GDHLRLEPLGHALVDPLVQGVEEVVRPLQLDVGVQRVALVEQVAEVGEGLDHEAGQAHGALVWRLPPS	18
SB19	1	MDEKTTGWRGGHVVEGLAGELEQLRARLEH**HPQ**GQREPLVQEVEDVDEGLVQDLHGVVAGLLDPVEKLLTDWFKKFKNVSKDCKMTFYLEMYDWSGGCHHH	19
SB20	1	MNEKTHCKLNFKVNIADWLAEFHGGGQGLLGRRDGVVQRLVDGVQERVERLDRDPGLGDLRLELHHRDHRLRLGGEHLLRDHPLEPDDHLVVGGLVWRLPPS	20

Fig. 3

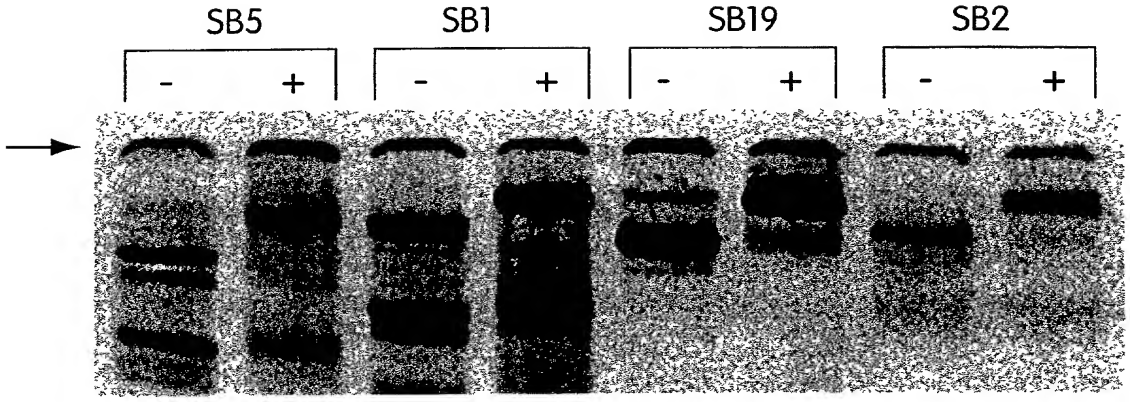

Fig. 4A

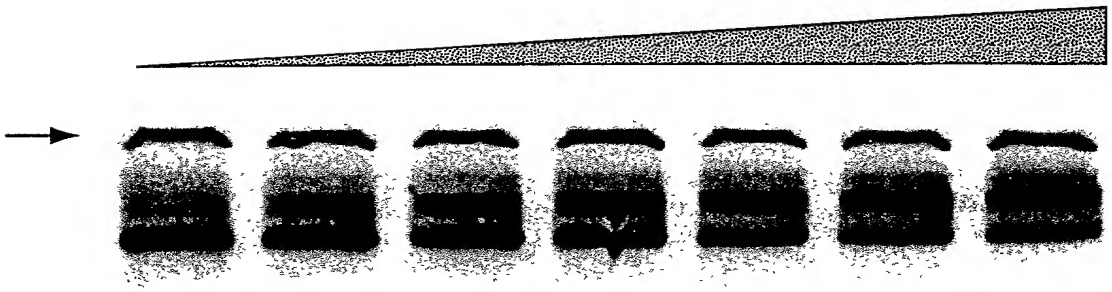

Fig. 4B

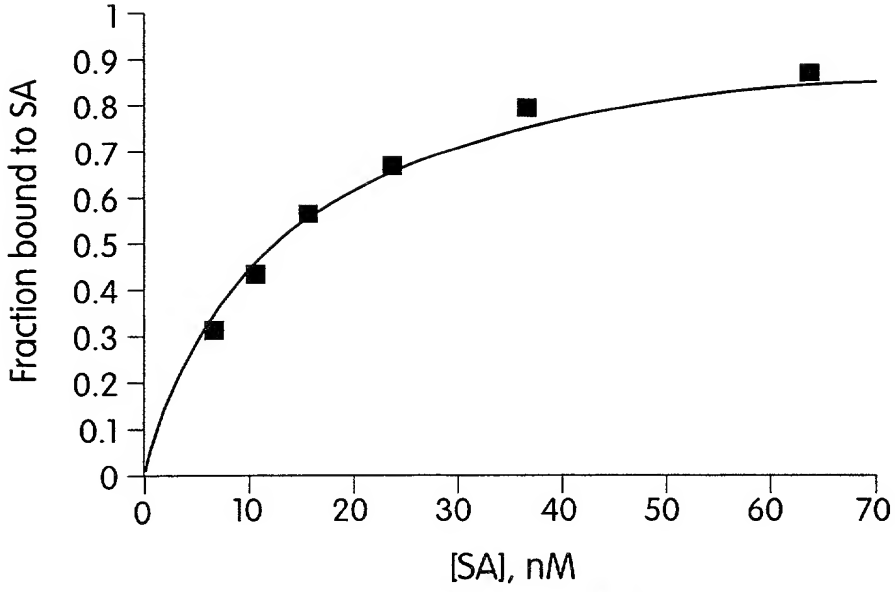

Fig. 4C

		% binding	SEQ ID NO:
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C1	MDEKTTGWRGGHVVEGLAGELEQLRARLEH**HPQ**GQREPLVQEVEDVDEGLVQDLHGVVAGLLDPVEKLLTDWFKKFKNVS MMSGGCKLG	**87**	22
C2	MDEKTTGWRGGHVVEGLAGELEQLRARLEH**HPQ**GQREPLVQEVEDVDEGLVQDLHGVVAGLLDPVE MMSGGCKLG	**88**	23
C3	MDEKTTGWRGGHVVEGLAGELEQLRARLEH**HPQ**GQREPLVQEVEDVDEGLVQ MMSGGCKLG	**89**	24
C4	MDEKTTGWRGGHVVEGLAGELEQLRARLEH**HPQ**GQREP MMSGGCKLG	**88**	25
M1	MDEKTTGWRGGHVVEGLAGELEQLRARLEH**HGA**GQREP MMSGGCKLG	**0.065**	26
N1	MD GHVVEGLAGELEQLRARLEH**HPQ**GQREP MMSGGCKLG	**69**	27
N2	MD EGLAGELEQLRARLEH**HPQ**GQREPLVQEVEDVDEGLVQDLHGVVAGLLDPVEKLLTDWFKKFKNVSKDCKMTFYLEMYDWSGGCKLG	**30**	28
N3	M ELEQLRARLEH**HPQ**GQREP MMSGGCKLG	**0.058**	29

Fig. 5

(SEQ ID No.: 37)

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Fig. 6A

CGCAAATTGTCGCGGCGATTAAATCTCGCGCCGATCAACTGGGTGCCAGCGTGGTGGTGTCGATGGTAGAACGAAGCGGC
GTCGAAGCCTGTAAAGCGGCGGTGCACAATCTTCTCGCGCAACGCGTCAGTGGGCTGATCATTAACTATCCGCTGGATGA
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Fig. 6A (continued)

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Fig. 6A (continued)

(SEQ ID No.:38)

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TMENAQKGEIMPNIPQMSAFWYAVRTAVINAASGRQTVDEALKDAQTNSSSGGSGSGMDEKTTGWRGGHVVEGLAGELEQ
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Fig. 6B

(SEQ ID No.:39)

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Fig. 6C

PCT/EP2004/011006

Fig. 7A

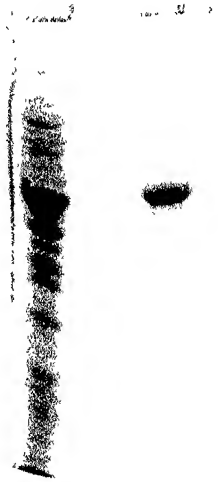
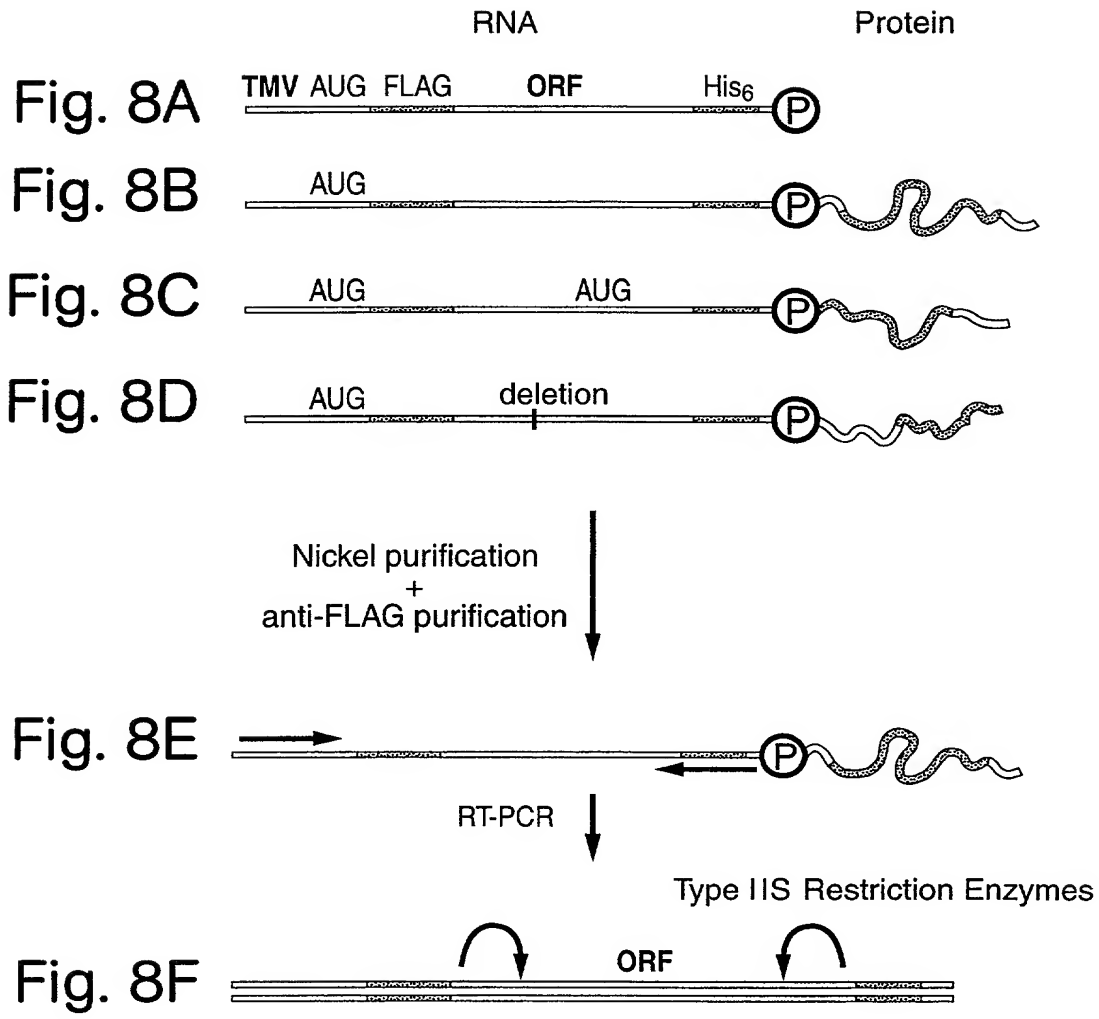


Fig. 7B

patent pending



(SEQ ID No.:40)

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GGTAGAACGAAGCGGCGTCGAAGCCTGTAAAGCGGCGGTGCACAATCTTCTCGCGCAACGCGTCAGTGGGCTGATCATTA

Fig. 9A

ACTATCCGCTGGATGACCAGGATGCCATTGCTGTGGAAGCTGCCTGCACTAATGTTCCGGCGTTATTTCTTGATGTCTCT
GACCAGACACCCATCAACAGTATTATTTTCTCCCATGAAGACGGTACGCGACTGGGCGTGGAGCATCTGGTCGCATTGGG
TCACCAGCAAATCGCGCTGTTAGCGGGCCCATTAAGTTCTGTCTCGGCGCGTCTGCGTCTGGCTGGCTGGCATAAATATC
TCACTCGCAATCAAATTCAGCCGATAGCGGAACGGGAAGGCGACTGGAGTGCCATGTCCGGTTTTCAACAAACCATGCAA
ATGCTGAATGAGGGCATCGTTCCCACTGCGATGCTGGTTGCCAACGATCAGATGGCGCTGGGCGCAATGCGCGCCATTAC
CGAGTCCGGGCTGCGCGTTGGTGCGGATATCTCGGTAGTGGGATACGACGATACCGAAGACAGCTCATGTTATATCCCGC
CGTTAACCACCATCAAACAGGATTTTCGCCTGCTGGGGCAAACCAGCGTGGACCGCTTGCTGCAACTCTCTCAGGGCCAG
GCGGTGAAGGGCAATCAGCTGTTGCCCGTCTCACTGGTGAAAAGAAAAACCACCCTGGCGCCCAATACGCAAACCGCCTC
TCCCCGCGCGTTGGCCGATTCATTAATGCAGCTGGCACGACAGGTTTCCCGACTGGAAAGCGGGCAGTGAGCGCAACGCA
ATTAATGTAAGTTAGCTCACTCATTAGGCACCGGGATCTCGACCGATGCCCTTGAGAGCCTTCAACCCAGTCAGCTCCTT
CCGGTGGGCGCGGGGCATGACTATCGTCGCCGCACTTATGACTGTCTTCTTTATCATGCAACTCGTAGGACAGGTGCCGG
CAGCGCTCTGGGTCATTTTCGGCGAGGACCGCTTTCGCTGGAGCGCGACGATGATCGGCCTGTCGCTTGCGGTATTCGGA
ATCTTGCACGCCCTCGCTCAAGCCTTCGTCACTGGTCCCGCCACCAAACGTTTCGGCGAGAAGCAGGCCATTATCGCCGG
CATGGCGGCCCCACGGGTGCGCATGATCGTGCTCCTGTCGTTGAGGACCCGGCTAGGCTGGCGGGGTTGCCTTACTGGTT
AGCAGAATGAATCACCGATACGCGAGCGAACGTGAAGCGACTGCTGCTGCAAAACGTCTGCGACCTGAGCAACAACATGA
ATGGTCTTCGGTTTCCGTGTTTCGTAAAGTCTGGAAACGCGGAAGTCAGCGCCCTGCACCATTATGTTCCGGATCTGCAT
CGCAGGATGCTGCTGGCTACCCTGTGGAACACCTACATCTGTATTAACGAAGCGCTGGCATTGACCCTGAGTGATTTTTC
TCTGGTCCCGCCGCATCCATACCGCCAGTTGTTTACCCTCACAACGTTCCAGTAACCGGGCATGTTCATCATCAGTAACC
CGTATCGTGAGCATCCTCTCTCGTTTCATCGGTATCATTACCCCCATGAACAGAAATCCCCCTTACACGGAGGCATCAGT
GACCAAACAGGAAAAAACCGCCCTTAACATGGCCCGCTTTATCAGAAGCCAGACATTAACGCTTCTGGAGAAACTCAACG
AGCTGGACGCGGATGAACAGGCAGACATCTGTGAATCGCTTCACGACCACGCTGATGAGCTTTACCGCAGCTGCCTCGCG
CGTTTCGGTGATGACGGTGAAAACCTCTGACACATGCAGCTCCCGGAGACGGTCACAGCTTGTCTGTAAGCGGATGCCGG
GAGCAGACAAGCCCGTCAGGGCGCGTCAGCGGGTGTTGGCGGGTGTCGGGGCGCAGCCATGACCCAGTCACGTAGCGATA
GCGGAGTGTATACTGGCTTAACTATGCGGCATCAGAGCAGATTGTACTGAGAGTGCACCATATATGCGGTGTGAAATACC
GCACAGATGCGTAAGGAGAAAATACCGCATCAGGCGCTCTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCGTTC
GGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAAC
ATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCC
TGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCC
CTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGC
GTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGA
ACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGC

Fig. 9A (continued)

"P" "E" "G" "P" " "P" "E" "G" "P"

CACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCT
AACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAG
CTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAG
GATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTC
ATGAACAAATAAAAACTGTCTGCTTACATAAACAGTAATACAAGGGGTGTTATGAGCCATATTCAACGGGAAACGTCTTGCT
CTAGGCCGCGATTAAATTCCAACATGGATGCTGATTTATATGGGTATAAATGGGCTCGCGATAATGTCGGGCAATCAGGT
GCGACAATCTATCGATTGTATGGGAAGCCCGATGCGCCAGAGTTGTTTCTGAAACATGGCAAAGGTAGCGTTGCCAATGA
TGTTACAGATGAGATGGTCAGACTAAACTGGCTGACGGAATTTATGCCTCTTCCGACCATCAAGCATTTTATCCGTACTC
CTGATGATGCATGGTTACTCACCACTGCGATCCCCGGGAAAACAGCATTCCAGGTATTAGAAGAATATCCTGATTCAGGT
GAAAATATTGTTGATGCGCTGGCAGTGTTCCTGCGCCGGTTGCATTCGATTCCTGTTGTAATTGTCCTTTTAACAGCGA
TCGCGTATTTCGTCTCGCTCAGGCGCAATCACGAATGAATAACGGTTTGGTTGATGCGAGTGATTTTGATGACGAGCGTA
ATGGCTGGCCTGTTGAACAAGTCTGGAAAGAAATGCATAAACTTTTGCCATTCTCACCGGATTCAGTCGTCACTCATGGT
GATTTCTCACTTGATAACCTTATTTTTTGACGAGGGGAAATTAATAGGTTGTATTGATGTTGGACGAGTCGGAATCGCAGA
CCGATACCAGGATCTTGCCATCCTATGGAACTGCCTCGGTGAGTTTTCTCCTTCATTACAGAACGGCTTTTTCAAAAAT
ATGGTATTGATAATCCTGATATGAATAAATTGCAGTTTCATTTGATGCTCGATGAGTTTTTCTAAGAATTAATTCATGAG
CGGATACATATTTGAATGTATTTAGAAAAATAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACCTGAAA
TTGTAAACGTTAATATTTTGTTAAAATTCGCGTTAAATTTTTGTTAAATCAGCTCATTTTTTAACCAATAGGCCGAAATC
GGCAAAATCCCTTATAAATCAAAAGAATAGACCGAGATAGGGTTGAGTGTTGTTCCAGTTTGGAACAAGAGTCCACTATT
AAAGAACGTGGACTCCAACGTCAAAGGGCGAAAAACCGTCTATCAGGGCGATGGCCCACTACGTGAACCATCACCCTAAT
CAAGTTTTTTGGGGTCGAGGTGCCGTAAAGCACTAAATCGGAACCCTAAAGGGAGCCCCCGATTTAGAGCTTGACGGGGA
AAGCCGGCGAACGTGGCGAGAAAGGAAGGGAAGAAAGCGAAAGGAGCGGGCGCTAGGGCGCTGGCAAGTGTAGCGGTCAC
GCTGCGCGTAACCACCACACCCGCCGCGCTTAATGCGCCGCTACAGGGCGCGTCCCATTCGCCA

Fig. 9A (continued)

(SEQ ID No.:41)

MGIEEGKLVIWINGDKGYNGLAEVGKKFEKDTGIKVTVEHPDKLEEKFPQVAATGDGPDIIFWAHDRFGGYAQSGLLAEI
TPDKAFQDKLYPFTWDAVRYNGKLIAYPIAVEALSLIYNKDLLPNPPKTWEEIPALDKELKAKGKSALMFNLQEPYFTWP
LIAADGGYAFKYENGKYDIKDVGVDNAGAKAGLTFLVDLIKNKHMNADTDYSIAEAAFNKGETAMTINGPWAWSNIDTSK
VNYGVTVLPTFKGQPSKPFVGVLSAGINAASPNKELAKEFLENYLLTDEGLEAVNKDKPLGAVALKSYEEELAKDPRIAA
TMENAQKGEIMPNIPQMSAFWYAVRTAVINAASGRQTVDEALKDAQTNSSSGGSGSGMDEKTTGWRGGHVVEGLAGELEQ
LRARLEHHPQGQREPGSGHHHHHHEF

Fig. 9B

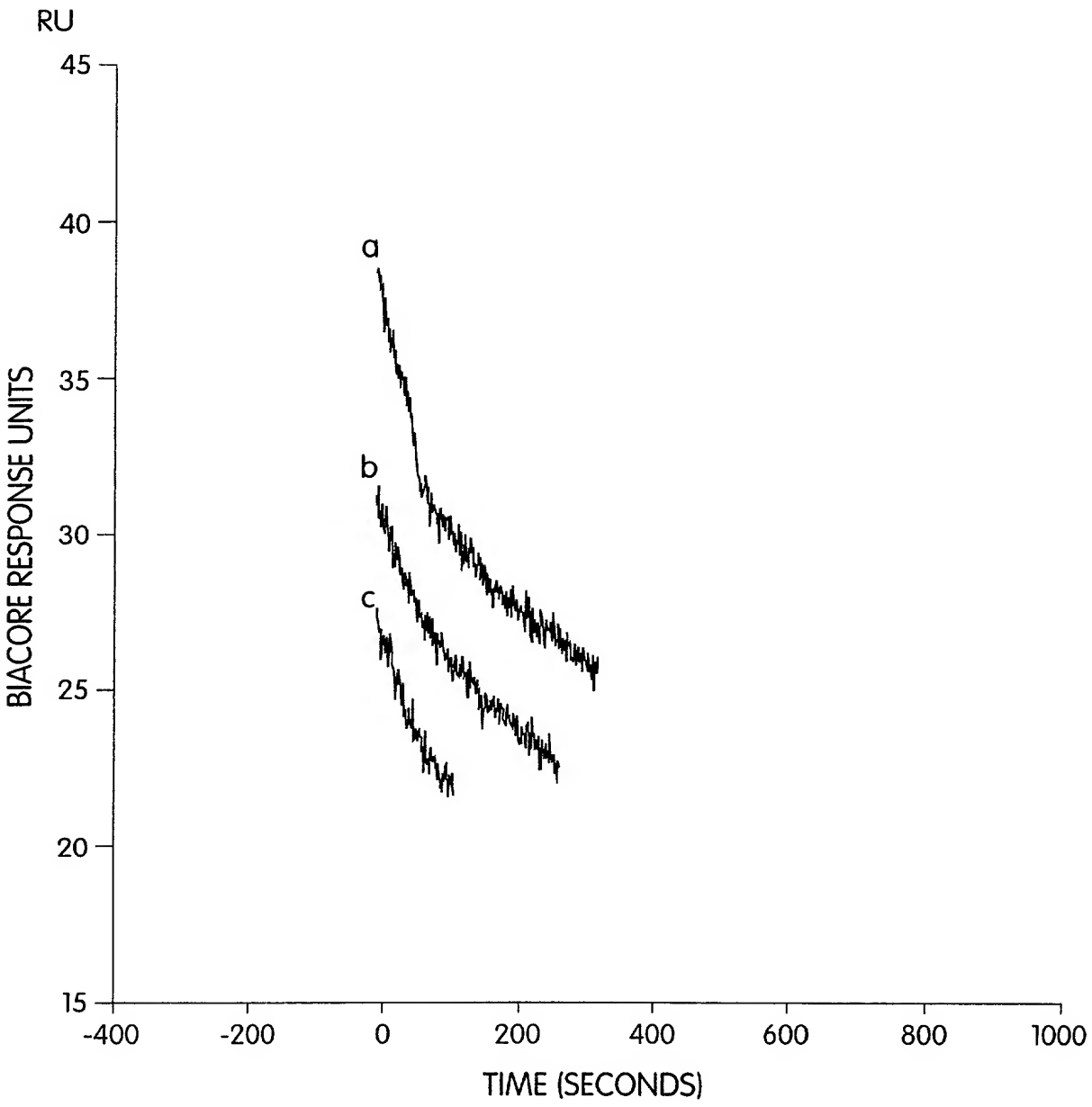
RU
45
40
35
30
25
20
15
BIACORE RESPONSE UNITS
a
b
c
-400
-200
0
200
400
600
800
1000
TIME (SECONDS)

Fig. 10A

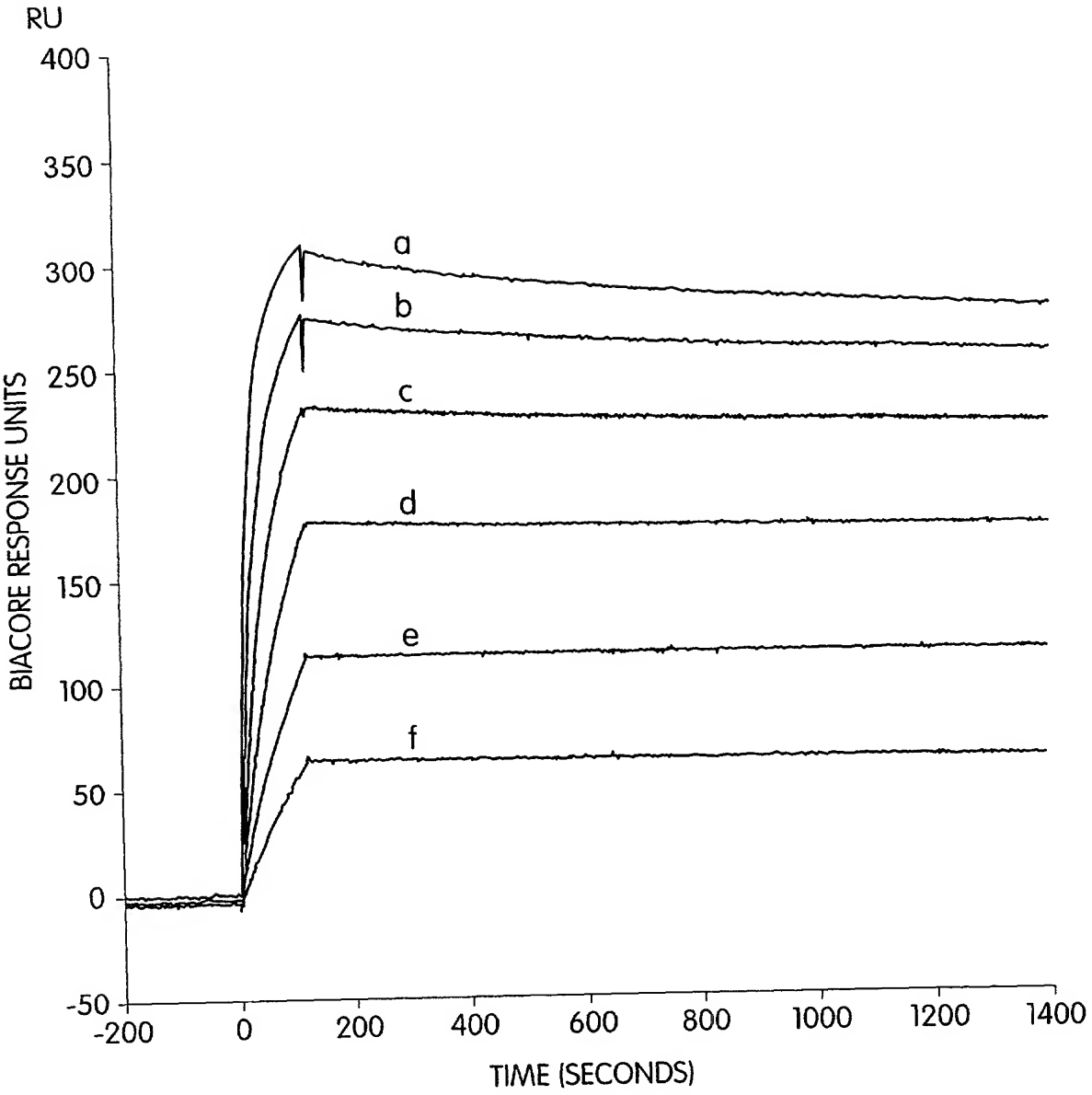
RU
400
350
300
250
200
150
100
50
0
-50
BIACORE RESPONSE UNITS
a
b
c
d
e
f
-200
0
200
400
600
800
1000
1200
1400
TIME (SECONDS)

Fig. 10B

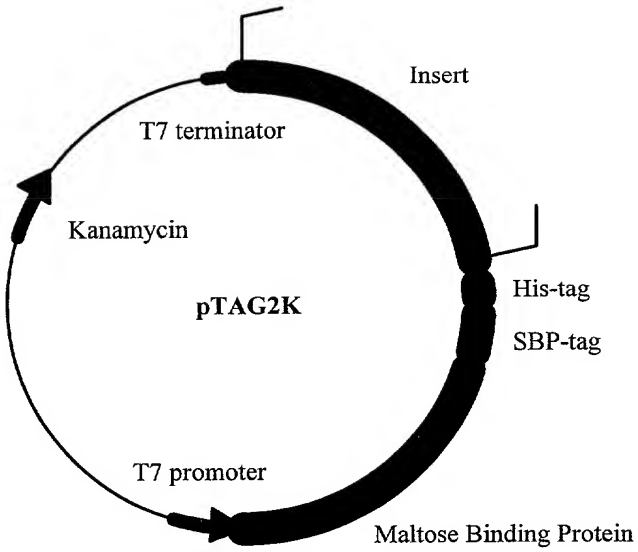
Insert
T7 terminator
Kanamycin
pTAG2K
His-tag
SBP-tag
T7 promoter
Maltose Binding Protein

Fig. 11

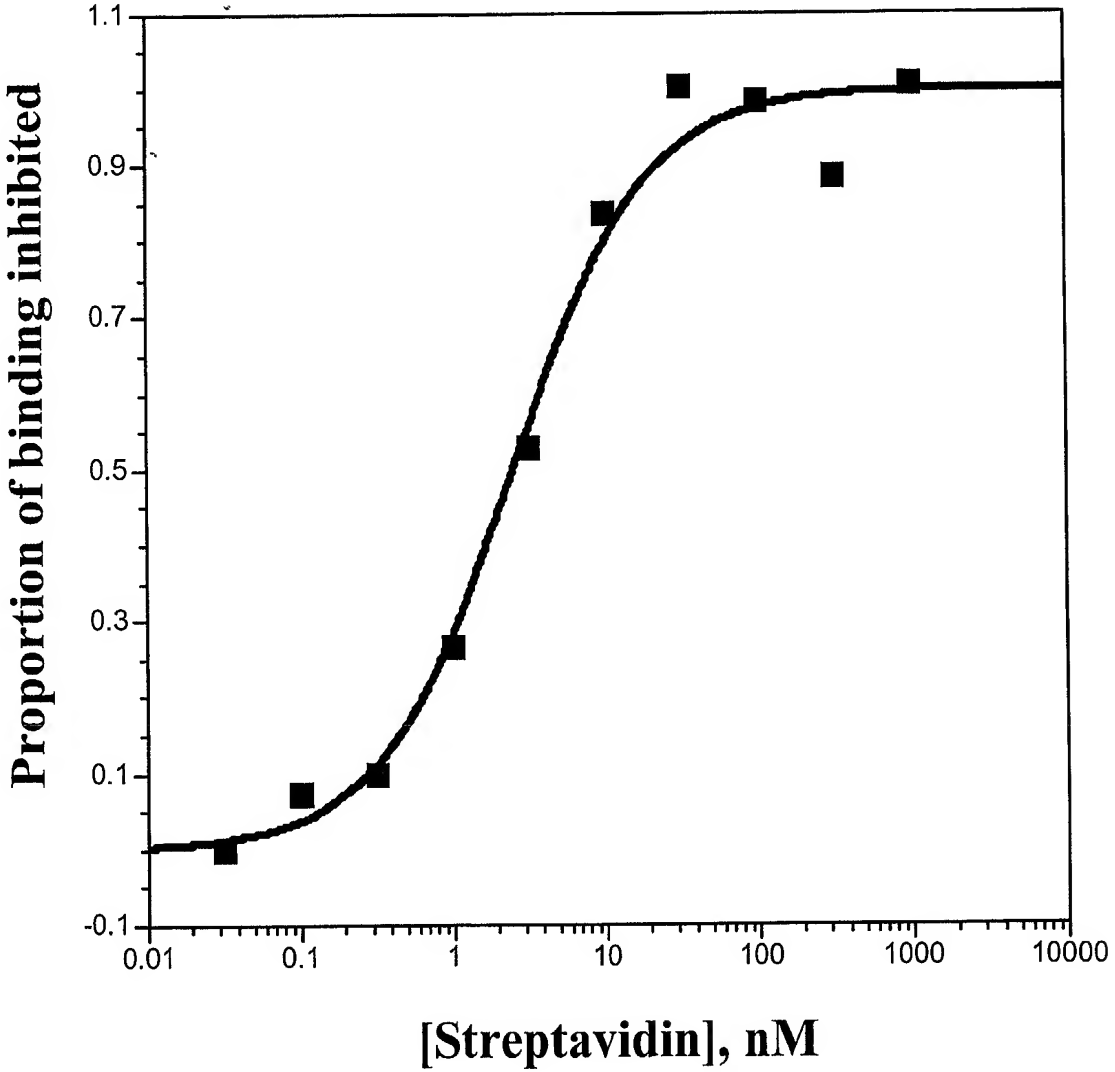
Proportion of binding inhibited
1.1
0.9
0.7
0.5
0.3
0.1
-0.1
0.01
0.1
1
10
100
1000
10000
[Streptavidin], nM

Fig. 12

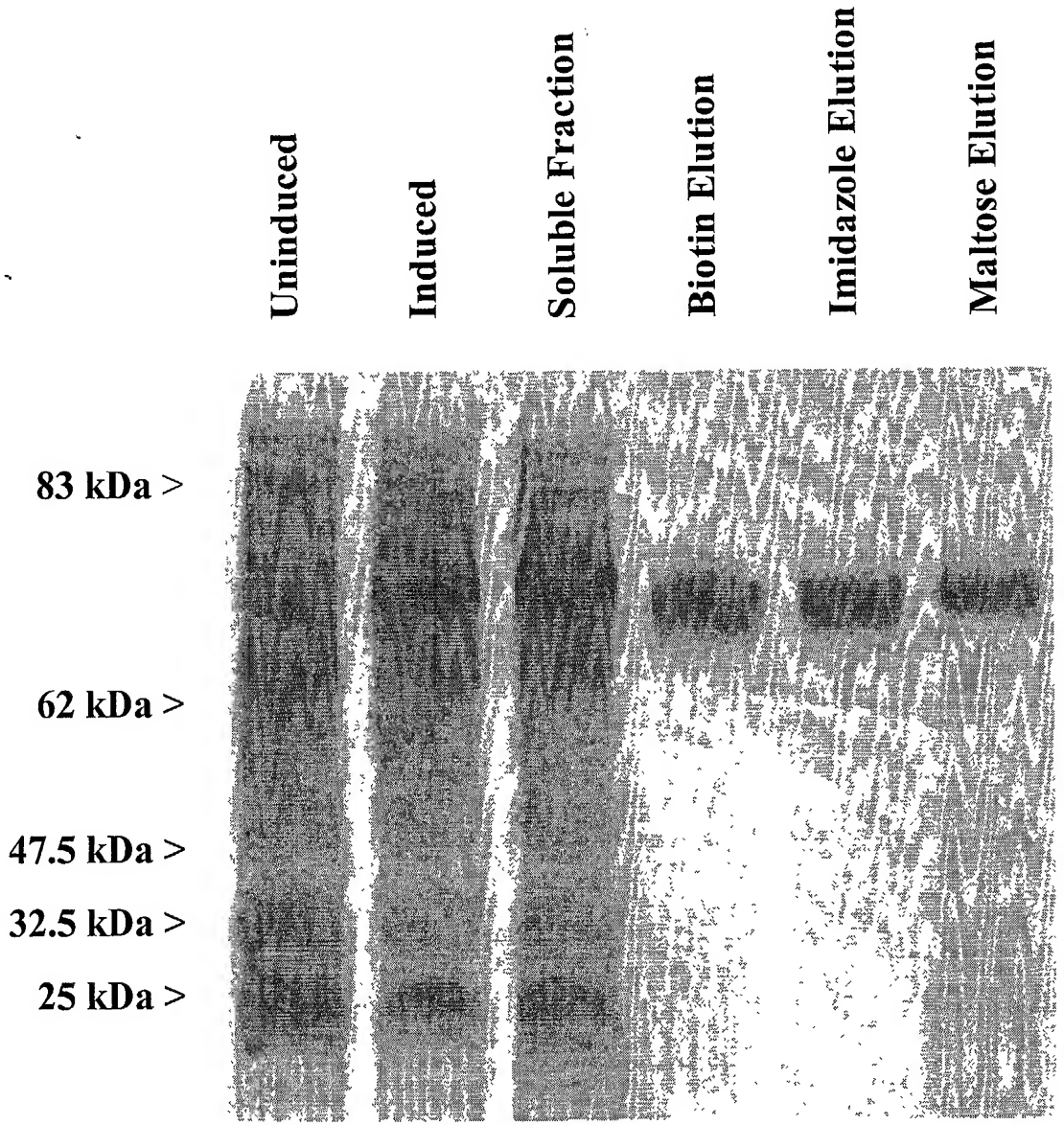
Uninduced
Induced
Soluble Fraction
Biotin Elution
Imidazole Elution
Maltose Elution
83 kDa >
62 kDa >
47.5 kDa >
32.5 kDa >
25 kDa >

Fig. 13

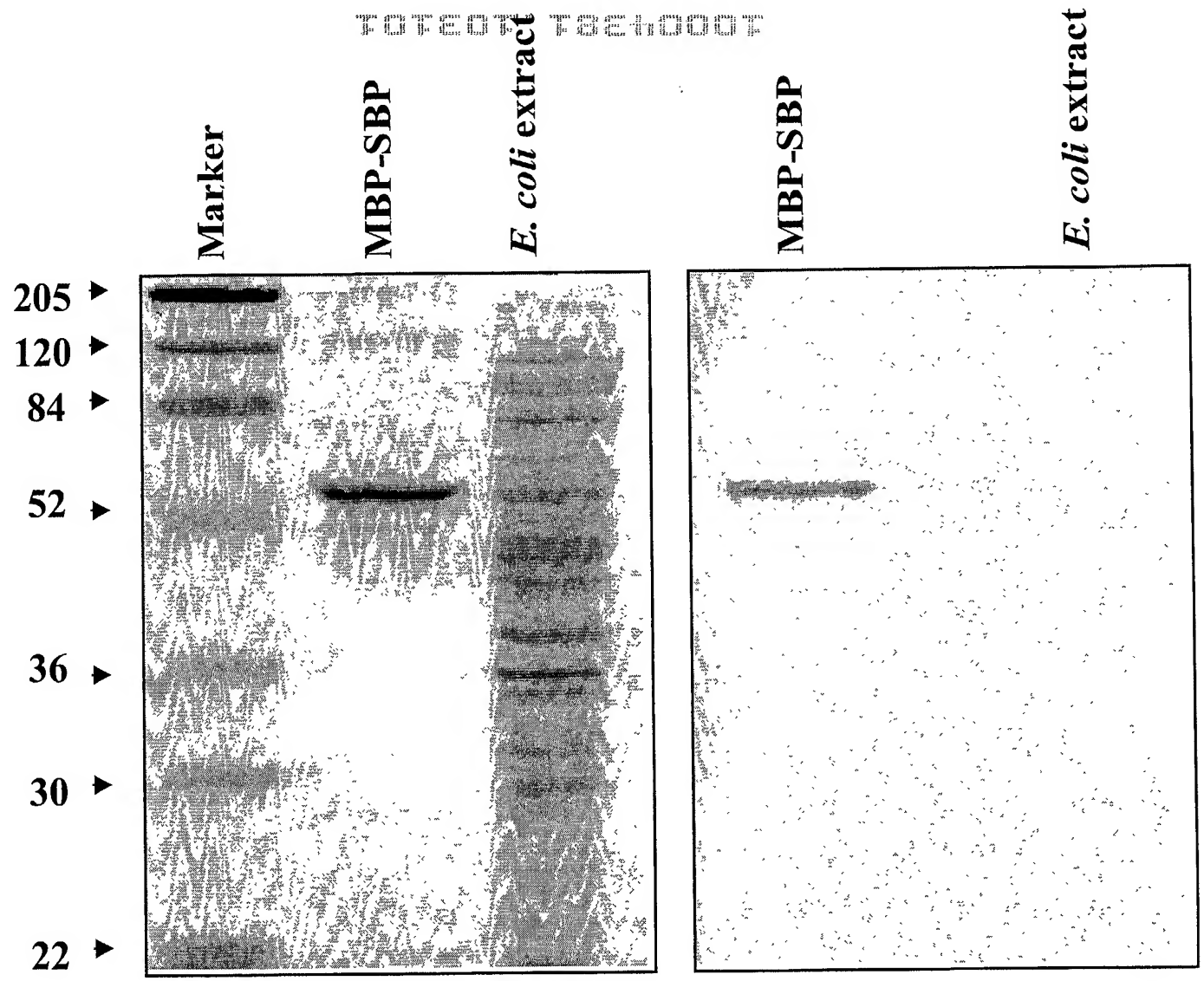
Marker
MBP-SBP
E. coli extract
MBP-SBP
E. coli extract
205
120
84
52
36
30
22
Total protein stain
Blot developed with SA-HRP

Fig. 14

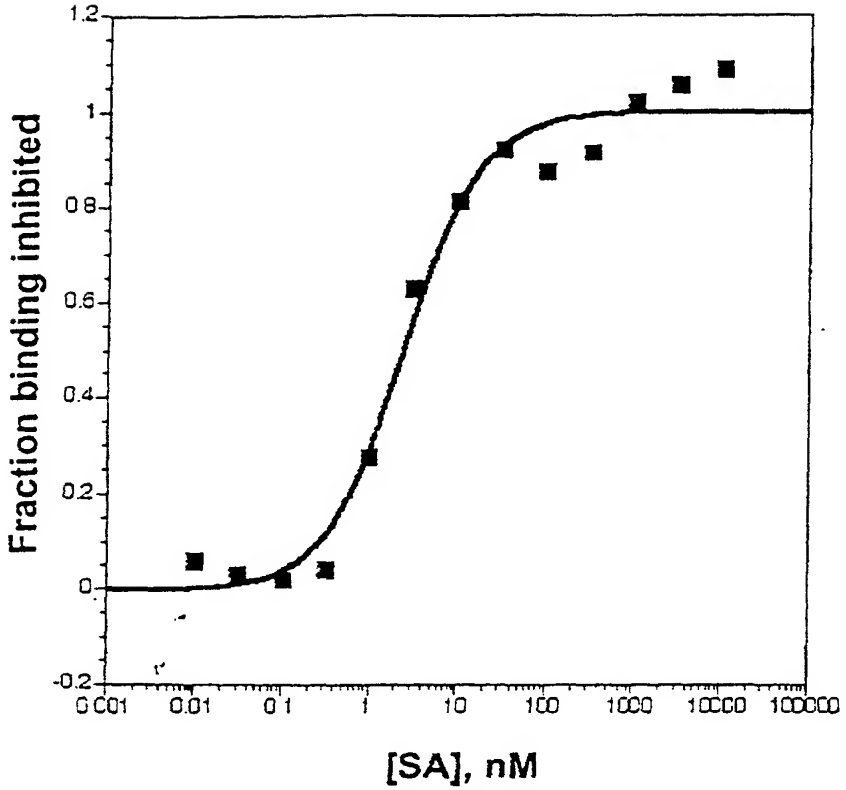
Fraction binding inhibited
1.2
1
0.8
0.6
0.4
0.2
0
-0.2
0.001
0.01
0.1
1
10
100
1000
10000
100000
[SA], nM

Fig. 15